REMARKS

Applicants respectfully request entry of amendments to claims 20-22 and cancel claim 23. Claims 1-19 and 26-28 are withdrawn, without prejudice or disclaimer. Support for the amendments can be found throughout the specification, including paragraphs [0043], [0045], [0047], [0051], [0057]-[0061], [0089], [0094], Examples 8 and 10-12, and the originally filed claims and, therefore, do not add new matter.

Applicants submit that pending claims 20-22, 24, 25, and 29 are in condition for allowance, and respectfully request that the claims as amended be entered.

Rejection Under 35 U.S.C. §112, First Paragraph

Claims 20-25 and 29 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement.

Applicants traverse the rejection as it might apply to the amended claims, including claims dependent therefrom, for the reasons given below.

The Office Action alleges, in pertinent part, "[w]hether sufficient claimed cells can be obtained in the artificial device inside or outside a subject and whether those claimed cells can provide sufficient liver specific biological activity to treat any liver disorder or disease in said subject were unpredictable at the time of the invention." Further, the Action states that "[the claims read on using the claimed cells for treating a subject having compromised liver function alone," including concerns about phenotypic/functional characteristics of the cells, and whether such cells would be able to provide sufficient liver specific activities for treating a subject having compromised liver function. The Action goes on to imply that the claims scope is not enabled with respect to the numerous different liver diseases or disorders. Moreover, as the claims encompass cells clonally derived from the cells of ATCC accession no. CRL-12461, such derivatives could deviate from the parental CRL-12461.

As the amended claims no longer recite "cells clonally derived from cells deposited as ATCC accession No. CRL-12461," this aspect of the rejection is rendered moot. With respect to the other issues, Applicants have amended the claims to recite that 1) cells are cultured on a surface in an extracorporeal bio-artificial liver device; 2) the device comprises a hollow fiber

cartridge formed from a material which has a pore size of about 0.1 μm to 0.3 μm, and wherein the cartridge is at least 1400 cm^2; and 3) the cultured cells interact with the blood to provide bio-artificial liver support for the subject.

Further, that the device as claimed functions as predicted is supported by a Declaration under 37 C.F.R. §1.132 that describes the results of a Phase I clinical device, which results show the biocompatibility, safety, and efficaciousness of an extracorporeal liver assist device (i.e., ELAD) comprising the cells as claimed. Moreover, the device has been proven to be effective.

Again, the specification clearly teaches 1) critical liver functions that must be considered (see, e.g., paragraph [0044]), 2) use of polarized aggregates in hollow cartridge devices (see, e.g., paragraph [0060]), including the devices themselves (see, e.g., paragraphs [0057]-[0060]), 3) cell density to achieve necessary function (see, e.g., paragraph [0060]), and 4) a specific disease to be treated (e.g., Fulminant hepatic failure (FHF), at paragraphs [0054] and [0055]), including that it is well known that subjects suffering from FHF have low albumin (see, e.g., http//homepage.mac.com/guitarbloke/Surgical_sieve/Hepatobiliary/Liver/Hepat_FHF.html), a specific protein that is produced by the cells as claimed (see, e.g., Example 4, Table 1).

Further, because the claims expressly recite a specific C3A cell type to be used in the device/methods (i.e., cell line deposited as ATCC accession No. CRL-12461, wherein the cells have a doubling time in serum-free medium which is less than about 70% of the doubling time in serum-free medium for C3A cells), the claims do not embrace "any and all" C3A derivatives. Moreover, the claims are enabled because the specification provides prediction of function based on tested and workable materials and designs of prosthetics which were well known in the art at the time the application was filed (see, e.g., paragraphs [0058]-[0059]), including that, as evidenced by the accompanying Declaration, the device as claimed functions as predicted.

Thus, one of skill in the art could practice the invention as claimed, in the absence of undue experimentation. For these reasons, Applicants respectfully request that the rejection, including as it may be applied to the amended claims, be withdrawn.

Rejection Under 35 U.S.C. §103

Claims 20 and 21 stand rejected under 35 U.S.C. §103(a), as allegedly being unpatentable over Spiering et al. in view of Price et al.

Applicants traverse the rejection as it might apply to the amended claims, including claims dependent therefrom, for the reasons given below.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First there must be some suggestion or motivation in the references themselves or in knowledge generally available to one of skill in the art, to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success. And, finally the prior art reference (or references when combined) must teach all claim limitations. The teaching or suggestion and reasonable expectation of success must both be found in the prior art and not in Applicants' disclosure. (See MPEP §706.02(j)).

Applicants submit that because the cited references would not result in cells having the properties as claimed, one of skill in the art would not be motivated to combine the reference teachings.

Review of Spiering et al. demonstrates that the device used comprises Hepatix C3A cells for an extracorporeal device. James H. Kelly, which is a co-author of the Spiering et al. Abstract describes the properties of these C3A cells in U.S. Pat. No. 5,290,684 (see Exhibit A, where ATCC No. CRL-10741 describes C3A [HepG2/C3A; derivative of HepG2]).

The cells of the present invention have the specific property of having a doubling time in serum-free medium (SFM) which is less than about 70% of the doubling time in serum-free medium for C3A cells. Thus, as the cells taught in Spiering et al. are C3A cells, and the cells of the present claims are cells of the CRL-12461 deposit, which are derived from C3A cells, the cells of the present claims **cannot** be the same as the hepatix C3A cells as taught by Spiering et al.

Further, Price et al. do not cure this deficiency. This is because, even if, *ad arguendo*, Price et al. do teach the use serum free media, this alone would not lead one of skill in the art to conclude that for "C3A cells in SFM it is inherent that the resulting clonal C3A cells would have a doubling time in SFM significantly less than the doubling time of the parent C3A cells in SFM

as claimed in the instant invention" (page 11, Office Action of May 9, 2006), since the effect of SFM on doubling time is not so predictable. Or put another way, based on the conclusion offered in the Action, culturing cells in SFM will **always** result in cells whose doubling time will be significantly less that the doubling time of the parent from which it is derived. Applicants submit that such a conclusion is simply incorrect.

For example, as a general rule, one of skill in the art would expect cells grown in SFM to have an *increased* doubling time, if the cells grow at all (see, e.g., Shapiro and Wagner In Vitro Cell Dev Biol (1988) 24(4):299-303; Exhibit B, which demonstrates that when H-35 rat hepatoma cells are grown in unsupplemented medium, the cells had a doubling time of 46 hours (in 10% FBS, the doubling time was 17 hours)). Even when the serum free medium was supplemented with transferrin and insulin, the growth rate only equaled the rate seen for 10% FBS. For other cells, the direct opposite may be seen, as such an effect of SFM cannot be known *apriori*. At best, the combination represents an example of "obvious-to-try," and it is axiomatic that "obvious to try" is not the standard under § 103. In re O'Farrell, 853 F.2d 894, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988).

Therefore, because the teachings of Spiering et al. would not result in cells *inherently* possessing the property as described for the cells of the CRL-12461 deposit when combined with the teachings of Price et al., one of skill in the art would not have an expectation of success, since the invention as claimed would not be achieved in view of such teachings. Therefore, one of skill in the art would not be motivated to combine such teachings.

Applicants submit that because there is no reasonable expectation of successfully achieving the invention as claimed, there is no motivation to combine the cited references, thus, no *prima facie* case for obviousness exists. For these reasons, Applicants respectfully request that the rejection, including as it might be applied against the amended claims, be withdrawn.

Conclusion

Applicants submit that pending claims 20-22, 24, 25, and 29 are in condition for allowance, or are in better condition for appeal. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this submission.

A check in the amount of $60.00 is enclosed to cover a One Month Petition for Extension of Time fee. The Commissioner is hereby authorized to charge any additional fees required by this submission, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number. A copy the Transmittal Sheet is enclosed.

Respectfully submitted,

Date: February 26, 2007

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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Cell Biology

ATCC® Number:	**CRL-10741™** Order this item	**Price:**	**$203.00**
Designations:	C3A [HepG2/C3A; derivative of Hep G2 (ATCC HB-8065)]	**Depositors:**	Baylor College of Medicine
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma		
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen;,complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein)		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

This material is cited in a U.S. and/or other Patent or Patent Application, and may not be used to infringe on the patent claims.

Related Cell Culture Products

Tumorigenic:	No, in immunosuppressed mice Yes, in semisolid medium
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,13 D7S820: 10 THO1: 9

	TPOX: 8,9 vWA: 17
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian
Comments:	C3A is clonal derivative of Hep G2 that was selected for strong contact inhibition of growth, high albumin production, high production of alpha fetoprotein (AFP) and ability to grow in glucose deficient medium. [22052] As the cells become confluent, there is a marked reduction in AFP secretion and an increase in albumin secretion. [22052] Gluconeogenesis activity is strongly oxgen dependent. [22052] The cells have nitrogen metabolizing activity comparable to perfused rat livers. [22052] There is no evidence of a Hepatitis B virus genome in this cell line. [22052]
Propagation:	**ATCC complete growth medium:** Minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate, 90%; fetal bovine serum, 10% **Temperature:** 37.0C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. ***Subcultivation ratio:*** *A subcultivation ratio of 1:3 to 1:6 is recommended* ***Medium renewal:*** *Twice per week*
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003 recommended serum: ATCC 30-2020 parental cell line: ATCC HB-8065
References:	22052: Kelly JH . Permanent human hepatocyte cell line and its use in a liver assist device (LAD). US Patent 5,290,684 dated Mar 1 1994

price will be applied to most cultures for nonprofit institutions in the United States and Canada. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

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1: In Vitro Cell Dev Biol. 1988 Apr;24(4):299-303.

Links

Growth of H-35 rat hepatoma cells in unsupplemented serum-free media: effect of transferrin, insulin and cell density.

Shapiro LE, Wagner N.

Department of Medicine, Montefiore Medical Center, Bronx, New York.

Serum-free tissue culture medium consisting of a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium is herein shown to support growth of Reuber H-35 cells over several days in culture. Cells were initially plated in serum containing DMEM medium for 3 h. After cell attachment, serum is removed and replaced with a serum-free 1:1 mixture of these two commercially available tissue culture media. The doubling time of cell growth in this unsupplemented serum-free medium was 46 h in lightly plated cultures over the first 5 d. The presence of transferrin (5 micrograms/ml) and insulin (3.3 nM) results in a cell doubling time of 17 h, which equaled the growth rate in medium containing 10% fetal bovine serum. In the absence of transferrin, growth rates in serum-free medium were correlated with the cell density of cultures. Conditioned medium from dense, serum-free cultures has growth-stimulating activity in recipient lightly plated cultures. This simple, serum-free culture medium will facilitate studies on the growth regulation of H-35 rat hepatoma cells.

PMID: 3284876 [PubMed - indexed for MEDLINE]